Production of Mushroom Mycelium

Harry Humfeld

Growing mushrooms has become an important industry in the United States. Of the estimated annual production of 62 million pounds, 20 million reach the consumer as fresh mushrooms, 18 million in cans, and 24 million as flavoring in soups.

Consumption might be even greater if production costs could be reduced. With the trend towards packaging prepared, ready-to-serve food products, the use of mushroom flavor in a number of such preparations doubtlessly will increase. The problem is to incorporate mushroom flavor without markedly increasing the cost of the product.

The mushrooms in the retail markets are really the fruits of a plant that grows as a mat of fine strands or threads on the organic material in the soil or in a mushroom bed. When conditions of temperature, moisture, and food supply are right, such a mat, or mycelium, periodically forms mushroom fruits near the surface of the soil. These are the familiar buttons and caps. Soon after they push up through the bed they are ready for harvest.

Although people over the world eat mushrooms of several species, in the United States only one species—*Agaricus campestris*—is grown commercially.

In 1947, I discovered that the vegetative, or underground, part (mycelium) of that species will grow under conditions of submerged propagation. The technique offers the possibility of greatly cutting the cost of a mushroom-flavored food material, although the physical form and texture usually associated with mushrooms must be sacrificed.

The principles of the method are based on the fact that when a liquid that contains the required chemicals and food supply is continuously stirred vigorously at the right temperature and mixed thoroughly with a constant supply of air, many micro-organisms reproduce in it with great rapidity. The method is used commercially in the production of baker's yeast and feed yeast. More recently it has been applied to the large-scale production of antibiotics, such as penicillin and streptomycin, as well as to the manufacture of citric and gluconic acids.

The mycelium proved to be not too particular about the medium on which it will grow, but it does have certain requirements. It seems now that a good commercial product can be grown on any medium that contains a suitable sugar and other essential nutrients, that does not contain an ingredient inhibitory or toxic to the growth of the mycelium, and that does not impart a characteristic flavor of its own during its use as a medium.

TO OBTAIN PURE-CULTURE mushroom mycelium, we either germinated mushroom spores or made tissue cultures of various parts of the mushroom. Then we grew the mycelium on an agar medium in order to maintain the pure cultures. The cultures may be inoculated into sterilized compost, grain, or tobacco stems, and allowed to grow to provide the material known in the trade as mushroom spawn. The spawn, when planted in mushroom beds, produces the mushrooms sold commercially.

For the submerged propagation of the mycelium, the procedure now in use consists in transferring some of the mycelium grown on agar in a test tube to 50 milliliters of sterile liquid medium in a 250-milliliter Erlenmeyer (conical) flask. The flasks are shaken on a machine in a room at 77° F. until a heavy growth of mycelium has developed. The time usually required is 5 to 6 days. This inoculum is then transferred to 2 liters of medium in fermentors made from Fernbach (spherical) flasks and incubated to maximum growth. This culture suspension is used as inoculum in the larger fermentors with a culture-medium capacity of 16 to 20 liters.

It has been necessary to consider variations in so-called strains of *Agaricus campestris*. Plant species, like animal species, have familylike variations. Therefore I have isolated and tested more than 40 cultures of strains of *Agaricus campestris*.

Among them I have found three that are well able to adapt themselves to growth in an agitated, aerated liquid medium. Each has characteristic mushroom flavor. Each can be distinguished from the others by a characteristic difference in flavor. Two of the strains were isolated from mushrooms of the white variety and one from the cream or brown variety of *Agaricus campestris*. All have certain characteristics in common. They grow more rapidly than the other isolations. The hyphae, or mycelial threads, are more slender. They produce an abundance of so-called secondary spores in the liquid medium. The secondary spores were first described by Albert Kligman in 1932 at Pennsylvania State College. He found them in old cultures on solidified (agar) medium. From observations up to the present time, it seems likely that the ability to produce the secondary spores may account for the ability of the strains to adapt themselves to submerged growth in a liquid medium.

The process also has been carried successfully through a semipilot-plant stage. That is, we have grown all three strains in 40-gallon batches in our pilot-plant fermentor. Each batch has produced 35 to 38 pounds of mycelial cake with good mushroom flavor.

In both the Fernbach and the larger fermentors, a rate of air flow of 1 liter of air a minute per liter of culture medium gives rapid growth. Lower rates give slower growth. Higher rates do not materially increase the growth rate.

We harvest when good growth and good mushroom flavor have been produced in the large fermentors by taking out half to three-fourths of the culture and adding an equal volume of fresh sterile culture medium. In this manner a number of consecutive harvests are obtained without contamination of the culture medium.

Immediately after harvesting, we separate the mycelium from the culture liquid by centrifuging. Then we resuspend the mycelium cake in water and again centrifuge.

We pack the mycelium into suitable cans and seal and sterilize the filled cans at 15 pounds of steam pressure for 20 minutes. In some cases we have frozen it and stored it in a freezer until used. Drying the mycelium, either by lyophilizing (drying under vacuum from the frozen state) or on a drum drier, has been tried, with some loss of flavor in both cases.

We have obtained good yields on media composed of juices pressed from various fruit and vegetable wastes, such as asparagus butts and pear waste. Our more recent investigations show that a desirable product is obtainable from media made from pear waste, a rice-bran extract, or beet molasses, and also from a synthetic medium containing dextrose and inorganic salts. Media containing asparagus juice or alfalfa juice seemed to impart the flavor of those constituents and were deemed less desirable.

Basic studies in progress in 1949 demonstrated that the essential nutrient requirements for the growth of the mycelium of *A. campestris* are com-

paratively simple. Sources of nitrogen utilized include ammonia, urea, peptone, monosodium glutamate, a mixture of amino acids, and probably a number of other nitrogen compounds. Most of the sugars tested, which included hexoses, pentoses, and disaccharides, give good growth. The polysaccharides (soluble starch and dextrin) also are suitable sources. However, a form of soluble cellulose (sodium carboxymethyl cellulose) cannot be fermented. Apparently a wide range of carbohydrates can serve as sources of energy for growth of mycelium of *A. campestris*.

We have determined approximate optimum concentrations for the various elements required and have developed a basal medium which contains the required amounts of sugar, nitrogen, phosphorus, potassium, magnesium, sulfur, calcium, and the trace elements, iron, manganese, zinc, and copper.

The development of this synthetic medium of know composition and the determination of the nutrient requirements and utilization of various nitrogen and energy sources aid in investigations on the suitability of various media made from fruit and vegetable wastes. If the composition of the press juice of a particular waste material is known, a preliminary forecast can be made on the suitability of the waste as a source of substrate for mushroom mycelium production.

During the continuous-fermentation tests in the Fernbach and in the larger (20-liter) fermentors, successive harvests were made as soon as enough mycelium had been produced to give a good yield. We discovered that when this procedure was followed, much of the characteristic mushroom flavor was lost. To assure the production of a good-flavored mycelium, it was found necessary to grow each stage of development of the culture until a full mushroom flavor had developed.

We are investigating the effects of rates of aeration, agitation, length of time of fermentation, and the composition of the medium on the development of the intensity of the mushroom flavor.

Our tests on the possibilities of spawning mushroom beds by spraying the liquid culture on the compost gave negative results. The mycelium grows very rapidly, forming a white mat over the surface of the compost bed overnight, and then disappears nearly as quickly. The hyphae apparently are unable to penetrate into the compost or are unable to compete with the other micro-organisms present. This method of seeding beds of compost, if successful, would help reduce the costs of the commercial grower who supplies the commercial trade with mushroom fruit caps.

We have grown these cultures in our laboratory on rye grain by the method used in making commercial grain spawn. When we used grain spawn to start beds in the customary commercial manner, the hyphae formed an excellent fluffy growth on the grain, but again were unable to grow and spread into the compost. It may well be that these strains are spontaneous mutants, which can reproduce themselves only vegetatively in pure culture medium.

Results of analyses of mushroom mycelium produced by submerged fermentation show a protein content (nitrogen x 6.25) of 49.1 percent, fat 3.1 percent, and mineral constituents, as ash, 8.1 percent, calculated on a moisture-free basis. The mycelium of the white variety contained, in micrograms per gram of dry mycelium, the following vitamins: Thiamine, 8.7; riboflavin, 47; niacin, 190. For the cream variety, the figures were 8.7, 90, and 290, respectively. Both showed only a trace of ascorbic acid. The results compare favorably with those for commercially grown mushrooms.

The centrifuged mushroom mycelium looks like a fresh yeast cake and contains about 25 percent solids, as compared with 10 to 12 percent solids in fresh mushrooms. The mycelium should be suitable for use in soups, gravies, and condiments, wherever it

is not essential that recognizable pieces of mushroom be present. It is likely, of course, that people will continue to want whole mushrooms, even though the mycelial cake may be made for flavoring.

The advantage of mushroom mycelium over fresh mushrooms would seem to be largely that of more economical production, although the assurance of a constant and reliable supply might also be an important factor.

After the production of the mycelium of *A. campestris* has been carried through the pilot-plant stage, the use of mycelium of other mushroom species known to have desirable flavor characteristics remains to be explored. The mushrooms of many edible species are now available only in limited quantities and only for short periods during the year. A reliable and constant source of supply of mycelium of desirable species would seem to offer possibilities for the development of palatable food materials. Other species might be suitable as sources of feeds of high nutritive value or for the production of enzymes, antibiotics, toxins, and other organic compounds.

Another direction that research might take is toward basic factors in the medium that might enhance or otherwise modify and improve the concentration and quality of the flavor. Flavor substances are very important commercially. They are complex chemically and constitute an important and interesting field for the agricultural chemist and microbiologist.

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CITRUS MOLASSES is no good with hot biscuits, but to Florida cattle it's ice cream and cake. On the big ranches of central Florida, cattle greedily slurp up the black, sticky molasses as it trickles from 1,000-gallon drums into feeding troughs, or they chew their bulky feeds with more gusto if the molasses has been poured over them to make a sort of hay à la mode. This use of citrus molasses is an answer to one waste-disposal problem.

About 60 percent of Florida's huge citrus crop was processed in 1950, and the refuse from the processing plants created a serious disposal problem. Citrus molasses has eased the problem somewhat.

Florida cattlemen, scientists, and processors joined in the effort to turn the waste into livestock feed. Scientists found that it could be converted into citrus pulp and a brownish-black sirup, which they called citrus molasses.

The University of Florida ran trials on the value of the two products as cattle feed. The results were satisfactory. The Florida Agricultural Experiment Station found that citrus feeds can contribute to thickness of flesh, glossiness of coat, and flow of milk, and add to general body tone of cattle. Florida produced about 180,000 tons of citrus pulp and 80,000 tons of citrus molasses in 1950. Most of the pulp went to dairy cattle; most of the molasses to beef cattle. The supply and demand are expanding rapidly.—*Fred P. Lawrence, Agricultural Extension Service, University of Florida.*

Commercial production of fruit in the United States, 1940–49 average

Fruit	Average production 1,000 tons	Proportion of total fruit crop Percent	Major areas of production
Oranges and tangerines	3,700	22	Central Fla., southern Calif.
Grapes	2,800	17	Calif., Great Lakes region.
Apples	2,600	16	Central Wash., Shenandoah Valley, upper N. Y., southwest Mich.
Grapefruit	2,000	12	Central Fla., Rio Grande Valley.
Peaches	1,500	9	Central Calif., Ga., southwest Mich.
Melons (including cantaloups)	1,300	8	Calif., Ga., Fla., Tex., S. C., Ind.
Pears	750	4	Central Calif., Pacific Northwest.
Plums (including prunes)	670	4	Do.
Lemons	510	3	Southern Calif.
Apricots	220	1	Central Calif.
Cherries	190	1	Western Mich., western N. Y., central Calif.
Strawberries	160	1	Southern States, Mich., Pacific Coast States, Mass.
Others [1]	270	2	Calif., Fla., Mass., Wash.
Total	16,670	100	

[1] Avocados, bush berries, cranberries, dates, figs, limes, olives, persimmons, pineapples, and pomegranates.

The four main types of fruit processing in the United States, 1940–49 average

Fruit	Proportion of total crop processed	Average quantity— Canned	Dried	Frozen	Crushed [1]	Major processing areas
	Percent	1,000 tons	1,000 tons	1,000 tons	1,000 tons	
Oranges	26	.....	.....	.....	950	Fla.
Grapes	79	20	1,030	7	1,150	Calif.
Apples	26	220	130	27	300	N. Y., Shenandoah Valley, central Wash., Calif.
Grapefruit	51	89	.....	.....	920	Rio Grande Valley, Fla.
Peaches	40	480	130	17	.....	Calif.
Pears	38	260	18	.....	5	Calif., Pacific Northwest.
Plums (including prunes)	73	30	450	5	3	Calif.
Lemons	32	.....	.....	.....	160	Do.
Apricots	71	64	83	9	.....	Do.
Cherries	52	65	.....	30	2	N. Y., Mich., Wis., Pacific Northwest.
Strawberries	21	1	.....	32	1	Pacific Northwest, Southern States.
Others [2]	72	62	93	31	10	Calif., Fla., Mass.
Total	41	1,291	1,934	158	3,501	

[1] Includes fruit used for making juices and for fermented products, brined (in the case of cherries) and some quantities used for preserves, jams, and jellies.

[2] Avocados, bush berries, cranberries, dates, figs, limes, olives, persimmons, pineapples, and pomegranates.

Prepared from U. S. Bureau of Agricultural Economics data.